# Method of Treating Rhinitis or Sinusitis

## Field of the Invention

The present invention is directed to methods of treating inflammation associated with rhinitis or sinusitis by intranasally delivering peptidases to a patient. The invention also encompasses therapeutic packages in which a peptidase is preloaded in a device designed for intranasally delivering drug.

## Background of the Invention

Rhinitis, an inflammation of the nasal mucosal membrane, is characterized by sneezing, rhinorrhea, nasal congestion, and increased nasal secretion. It is often accompanied by sinusitis, an inflammation of the sinuses. When these conditions persist for a period of more than three weeks, they are termed "chronic." More than 37 million Americans, particularly those with allergies or asthma, suffer from these conditions, making them the most common chronic medical problems in the United States.

Chronic "rhinosinusitis" or sinusitis is difficult to treat successfully. In general, treatment consists of a combination of antibiotics and decongestants or antihistamines. In addition, steroid nasal sprays are commonly used to reduce inflammation. For patients with severe chronic sinusitis, oral steroids, such as prednisone, may also be prescribed. However, the long-term safety of steroid administration, especially in children, is not fully understood and oral steroids often have significant side effects. When drug therapy fails, surgery is usually the only alternative.

The mucosal tissue lining the nasal and sinus passages is densely packed with sensory neurons (Alving, *et al.*, *Cell Tissue Res. 264*:529-538 (1991); Saria, *et al.*, *Am. Rev. Respir. Dis. 147*:1330-1335 (1988)). When activated, these neurons release a variety of bioactive peptides that contribute to inflammation by causing vasodilation, stimulating mucosal gland secretion, and promoting infiltration by inflammatory mast cells, lymphocytes and eosinophils (Stead, *et al.*, *Immunol. Rev. 10*:333-359 (1987); Mygind, *et al.*, *Eur. J. Respir. Dis. 64(S128)*:1-379 (1983)). Included among the released bioactive peptides are substance P, calcitonin-gene related peptide, neuropeptide Y and vasoactive intestinal peptide (Lundblad, *et al.*, *Acta. Physiol. Scand. 529*:1-42 (1984)). Means for controlling the activity

of these peptides should provide an effective treatment for the inflammation associated with both rhinitis and sinusitis.

**Summary of the Invention**

The present invention stems from the discovery that dipeptidyl peptidase IV, an exopeptidase that cleaves Xaa-Pro dipeptides from the N-terminus of polypeptides, is present in human nasal mucosa at levels that are inversely related to inflammation. Thus, low levels of dipeptidyl peptidase IV are associated with a high density of inflammatory cells, and high levels of dipeptidyl peptidase IV are associated with a low density of inflammatory cells. This is important because dipeptidyl peptidase IV degrades peptides that contribute to the pathophysiology of rhinitis, sinusitis and asthma (Mentlein, *et al.*, *Regul. Peptides 49*:133-144 (1993); Heymann, *et al.*, *FEBS Lett. 91*:360-364 (1978); Beauvais, *et al.*, *Fum. Infect. Immun. 65*:3042-3047 (1997)). Among the inflammation-related peptides cleaved are NPY, SP, and desArg1 bradykinin. Based upon these observation and further experiments, the concept has been developed that the intranasal administration of dipeptidyl peptidase IV can reduce inflammation in the mucosal tissue that lines both the nasal cavity and sinuses. Other proteases that possess the same proteolytic activity should also produce a positive therapeutic effect. These proteases include quiescent cell proline dipeptidase (Underwood, *et al.*, *J. Biol. Chem. 274*:34053-34058 (1999)), dipeptidyl peptidase 8 (Abbott, *et al.*, *Eur. J. Biochem. 267*:6140-6150 (2000)), and attractin (Duke-Cohan, *et al.*, *Proc. Nat'l. Acad. Sci. U.S.A. 95*:11336-11341 (1998)).

In its first aspect, the invention is directed to a method of treating a patient for inflammation of the nasal or sinus mucosa. The method involves intranasally administering a therapeutically effective amount of a peptidase (preferably an exopeptidase) that cleaves at Xaa-Pro residues, where Xaa represents any of the 20 amino acids commonly found in animals. A "therapeutically effective" dose is defined as an amount sufficient to produce a significant reduction in inflammation as evidence by either a reduced number of inflammatory cells in mucosal tissue or by a significant improvement in one or more symptoms associated with the inflammation. For example, in the case of rhinitis or sinusitis, a therapeutically effective dose would be a sufficient amount to produce a significant reduction in sneezing, coughing, sinus-related headaches, nasal obstruction, mucosal secretion, or other discomfort associated with these conditions. Inflammatory cells include mast cells, lymphocytes and eosinophils. In general, it is expected that a therapeutically effective dose

for any of the proteases used will be between 1 microgram and 1 milligram and, typically, between 5 micrograms and 500 micrograms.

The preferred peptidase for use in the method is dipeptidyl peptidase IV. Other peptidases that can be used include quiescent cell proline dipeptidase; dipeptidyl peptidase 8, and attractin. In each case, it is the human form of the peptidase that is preferred. However, peptidases from other species (*e.g.*, that secreted by *Aspergilus Fumigatus*, see Examples section) may also be used provided that they have the ability to cleave at the Xaa-Pro sequence. Although the method will work for rhinitis and sinusitis caused by any disease or condition, it is expected that the most common causes will be allergies or asthma.

In another aspect, the invention is directed to a therapeutic package in which a device for intranasally delivering drug to a patient is preloaded with a solution or powder containing one or more of the peptidases described above. The invention is compatible with any intranasal delivery device (including encapsulated dosage forms) and with any of the numerous compositions that have been described for delivering drugs by means of the nasal cavity. When liquid compositions are used in the device, it is expected that peptidase will be present at a concentration of between 1 μg/ml and 10 mg/ml, and more typically, at a concentration of between 10 μg/ml. and 1 mg/ml.

The invention also encompasses the concept that SP is particularly important in causing inflammation in lung and nasal mucosa. Any method that reduces the local activity of this peptide should be useful in the treatment of rhinitis or sinusitis. A reduction in activity may be accomplished either using a peptidase that degrades SP (*e.g.*, one of the peptidases described above) or by administering an agent that inhibits the binding of SP to the NK1 receptor (see Examples section).

**Detailed Description of the Invention**

**A. Preparation of Peptidases**

The present invention is directed to treatment methods which utilize peptidases that have the common characteristic of cleaving at Xaa-Pro sites. These may be purchased commercially or obtained using any of the procedures described in the relevant literature. For example, the gene corresponding to the peptidase can be isolated and used for recombinant protein production. Especially preferred peptidases, along with references

relevant to their isolation and recombinant production, are: human dipeptidyl peptidase IV, shown herein as SEQ ID NO: 1 (Misumi, *et al.*, *Biochim. Biophys. Acta 15*:1131 (1992); Darmoul, *et al.*, *J. Biol. Chem. 267*:4824-4833 (1992); Abbott, *et al.*, *Immunogenetics 40*:331-338 (1994)); human quiescent cell proline dipeptidase, shown herein as SEQ ID NO: 2 (Underwood, *et al.*, *J. Biol. Chem. 274*:34053-34058 (1999)); human attractin, shown herein as SEQ ID NO: 3 (Duke-Cohan, *et al.*, *Proc. Nat'l. Acad. Sci. USA 95*:11336-11341 (1998); Nagase, *et al.*, *DNA Res. 5*:31-39 (1998)); and human dipeptidyl peptidase 8, shown herein as SEQ ID NO: 4 (Abbott, *et al.*, *Eur. J. Biochem. 267*:6140-6150 (2000)). In addition to being made recombinantly, these proteins can be synthesized using methods that are well-known in the art.

**B. Making of Pharmaceutical Compositions**

Compositions for intranasally delivering peptidases can be made in accordance with methods that are standard in the art (see, *e.g.*, Remington's Pharmaceutical Sciences, 16th ed. A. Oslo Editor, Easton Pa (1980)). Enzymes will typically be prepared in admixture with conventional excipients. Suitable carriers may include, but are not limited to: water; salt solutions; alcohols; vegetable oils; polyethylene glycols; gellatin; carbohydrates such as lactose, amylose or starch; talc; hydroxymethylcellulose etc. The pharmaceutical preparations can be sterilized and, if desired, mixed with auxiliary agents such as preservatives or stabilizers. The invention is compatible with any of the numerous compositions that have been disclosed in the art for nasal delivery including those in: U.S. 6,054,462; U.S. 4,946,870; U.S. 5,897,858; and U.S. 4,476,116. The concentration of peptidase present can vary over a wide range. Typically, in liquid formulations, peptide should be present in an amount of between 1 μg/ml and 10 mg/ml and, more commonly, at a concentration of between 10 μg/ml and 1 mg/ml.

***Treatment Methods***

The present invention is directed to methods for treating inflammation present in the mucosal lining of the nasal or sinus passages. It depends upon the direct administration of sufficient peptidase to proteolytically cleave peptides known to contribute to inflammation. The total dosage of peptidase to be administered to a patient should be at least the amount required to achieve this objective as reflected by a reduction or elimination of symptoms associated with inflammation. For example, a patient being treated for rhinosinusitis should receive sufficient compound to reduce or eliminate the frequency or intensity of sinus

headache, reduce coughing, congestion, sneezing, respiratory obstruction or other discomforts associated with the condition.

In general, a patient may begin by self-administering a relatively small dose of compound and then repeat administration as necessary. For example, a patient may begin by administering 0.1 mg per day and then increase the dosage upward using changes in inflammation-related symptoms as a guide. Typically, it is expected that patients will receive a daily dose of between 1 μg and 1 mg per day, and, more typically, between 5 μg and 500 μg. Daily dosages may be provided in either a single or multiple regimen with the latter being generally preferred. These are simply guidelines, since the actual dose will be determined by the patient and their physician based upon a variety of clinical factors.

***Therapeutic Packages***

In addition to the pharmaceutical compositions described above, the invention includes therapeutic packages for the intranasal delivery of the compositions. A therapeutic package is comprised of a device designed for the intranasal inhalation of medication which has been preloaded with a pharmaceutical composition containing one or more of the peptidases described above. In general, spray devices are preferred, such as those disclosed in U.S. 6,145,703; WO 95/00195; U.S. 5,307,953; EP 0388651; U.S. 4,017,007; U.S. 5,301,846; U.S. 3,176,883; or U.S. 4,286,735. Devices for administering powders or nasal drops may also be used. When the pharmaceutical composition is in the form of a solution, it is expected that peptidase should generally be present at a concentration of between 1 μg/ml and 10 mg/ml, and, more typically, at a concentration of between 10 μg/ml and 1 mg/ml.

# Examples

## I. Materials and Methods

*Recombinant Dipeptidyl Peptidase IV (DPPIV)*

A soluble form of DPPIV secreted from *Aspergilus Fumigatus* has been previously characterized and was used in the present experiments (Beauvais *et al.*, *Infection Immun. 65* 3042-3047 (1997)). The enzyme has an apparent molecular weight of 95 kDa. It was expressed in the yeast *Pichia Pastoris* and purified to more than 99% purity as assessed by electrophoresis and gel-filtration to reach a specific activity of 40 units/mg of protein. SP (1.8 μg) co-incubated with 0.016 μg of DPPIV for ten minutes at 37°C is degraded to SP5-11 as

identified by mass-spectrometry. If an excess of SP is added (3.5 μg) a partial of digestion of the peptide is observed.

*Patients*

Forty-five patients, 23 males and 22 females, suffering from nasal obstruction, rhinorrhea and headaches for more than eighteen months were included in the study. Pre-operative rhinoscopy revealed septal deviation associated with concha bullosa of the middle turbinate. All patients underwent septoplasty and partial middle turbinectomy under endoscopic control with general anesthesia. The age range was 14 to 64 years with the average patient being 39 years of age. Patients with allergy, nasal polyps or tumors were excluded.

*Tissue Processing*

Samples of middle turbinate mucosa from patients undergoing partial turbinectomy were fixed immediately in ice cold acetone with 2 mM phenyl methyl sulphonyl fluoride and 20 mM iodoacetamide and incubated overnight at -20°C. Biopsies were embedded in glycol methacrylate resin and allowed to polymerize overnight at 4°C.

*Antibodies*

The following monoclonal antibodies were used: CD26 (clone BA5, DAKO) directed against DPPIV protease, diluted 1:20; CD1A (Biogenex) for dendritic cells, diluted 1:20; CD31 (DAKO) recognizing the adhesion molecule PECAM on endothelial cells, diluted 1:20; and the polyclonal antibody CD3 (DAKO) directed against T cells and used at a dilution of 1:20.

*Immunohistochemical Staining*

Serial sections, 2 mm thick, were cut using a Reichert-ung microtome equipped with a glass knife. Immunohistochemical staining was performed using the streptavidin biotin-peroxidase method with aminoethyl-carbazole (AEC) as substrate.

*Quantification of Inflammation in Nasal Biopsies and Intensity of Symptoms*

Mucosal samples of the middle turbinate from both sides (N=90) were fixed in formaldehyde and dehydrated, embedded in paraffin, and colored by haematoxylin-eosin. They were then examined under a Zeiss microscope at 40X magnification. Histological

analysis included defining the integrity of the pseudo-stratified columnar epithelium, noting the presence or absence of edema and quantifying the number of inflammatory cells within the submucosa. This was accomplished using a scale graded from 0 to +++, where 0 meant no inflammatory cells and +++ represented abundant inflammatory cells. Using the Rank Spearman correlation test, the correlation between DPPIV and the degree of inflammation of the nasal mucosa was examined. The intensity of nasal obstruction, rhinorrhea and headache was recorded by means of a visual analog scale graded from 0 to 5, where 0 corresponds to the absence of symptoms and 5 corresponds to severely intense symptoms. Nasal airway resistance was recorded by means of anterior rhinomanometry (rhinotest).

*The Determination of DPPIV Activity in Human Mucosa Biopsies*

DPPIV activity was determined according to Scharpé, *et al.* (*Clin. Chim. Acta 195*: 125-132 (1990)) with the following modifications: nasal biopsies were sonicated in the presence of 0.5 ml of 100 mM Tris-HCl, pH 8, for 2 minutes on ice using a Branson sonifier (output 4) and centrifuged for 10 minutes at 15,000 rpm in a microfuge at 4°C. The supernatant was recovered and the pellet was treated with 0.5 ml of 100 mM Tris-HCl, pH8, containing 2% Triton X100. The suspension was vortexed for one minute and centrifuged for 10 minutes at 15,000 rpm in a microfuge at 4°C. The supernatant was recovered, pooled with the one obtained previously, and stored at -20°C. DPPIV activity was determined on 1, 2.5 and 5 μl of supernatant fluorometrically using Gly-Pro-AMC (Novabiochem) at 5 mM final concentration for 60 minutes at 37°C under agitation in an Eppendorf thermomixer in 25 μl of 100 mM Tris-HCl, pH 8. The reaction was stopped by the addition of 2.5 μl of pure acetic acid. The incubation mixture was recovered in 3 ml of water. A blank value was obtained by incubating the substrate in the absence of enzyme and a standard curve was determined using AMC fluorescence measurement on a fluorometer. The DPPIV activities were standardized based on wet tissue weight and specific activities expressed as pmoles of substrate converted per mg of tissue per minute.

*Experiments in the Pig In Vivo*

Experiments were performed on pigs of both sexes (body weight 18-25 kg). All animals were premedicated with atropine (0.05 mg/kg) and ketamine (20 mg/kg i.m.). They were anesthesized with thiopentone (5 mg/kg i.v.). After tracheostomy, animals were intubated and artificially ventilated by a volume regulated ventilator. During surgery, animals were given a continuous i.v. infusion of Ringer's solution, pancuronium bromide (0.25

mg/kg) and heparin. Each experiment lasted approximately 8 hours. Catheters were placed in the femoral artery for systemic blood pressure and heart rate monitoring and in the femoral vein for thiopentone, heparin and fluid administration (300 ml/hr). The contralateral femoral vein was canulated for blood sampling. Surgical preparation of the internal maxillary artery was done in accordance with Lacroix (*Acta Physiol. Scand. 136*:1-63 (1989)). Selective recording of the nasal arterial blood flow was performed with a Transonic flow probe (probe 2.4RB143) of 2.4 mm diameter placed around the sphenopalatine artery. The flow probe was connected to a T202S ultrasonic blood flowmeter. All the arterial branches situated downstream of the flow probe were ligated and cut except for the superficial temporal artery which was cannulated with a PE 90 catheter for infusions or injections.

Variations in the area under the curve (AUC) of the sphenopalatine artery vascular resistance (Vr), derived from both mean arterial blood pressure and systemic blood flow curves, were analyzed over time. Durations of the responses of the vascular resistance were compared and all results were expressed in percent of baseline. Vascular parameters were recorded simultaneously using a 6 pen trace recorder.

All animals were intranasally administered DPIV (50 μg, 26.5 pmoles/kg), subjected to sympathetic nerve stimulation (SNS, 15V, 5 ms, 10 Hz for 2 minutes) and then infused with histamine (0.1-25 μg), capsacin (0.01-25 μg), bradykinin (0.001-10 μg), SP or its C-terminal fragment SP 5-11, DPPIV, or NK1 antagonist L733060 in the superficial temporal artery under controlled conditions. In each case, the vascular response of the animal was measured. This entire procedure was then repeated after local i.a. pretreatment with the alpha-adrenergic receptor antagonist phenoxybenzanime (1 mg/kg).

*Vascular Responses to DPPIV in Domestic Pigs In Vivo*

The basal blood flow in the internal maxillary artery of the pig under control conditions was 4.92 +/- 0.7 ml $min^{-1}$ $kg^{-1}$. After section of the sympathetic nerves on the left side, the homolateral nasal arterial flow was 6.1 +/- 0.26 ml $min^{-1}$ $kg^{-1}$ (representing a 19.34% +/- 4.3% increase). The mean arterial blood pressure (MAP) was not modified by the section of the sympathetic nerves. Electrical stimulation of the sympathetic nerve induced a frequency-depended increase of the SVR whereas the MAP was not significantly modified. Sympathetic nerve stimulation at 10 Hz for 5 minutes reduced the blood flow in the maxillary artery from 4.92 +/- 0.7 ml $min^{-1}$ $kg^{-1}$ to 1.40 +/- 0.47 ml $min^{-1}$ $kg^{-1}$, representing an increase

in SVR of 71.5% and lasting more than 6 minutes. The i.a. infusion of phenoxybenzamine induced a MAP reduction of 10 +/- 2%. The basal blood flow in the sphenopalatine artery, the heart rate and the MAP were not significantly affected by the administration of exogenous dipeptidyl peptidase IV.

*Statistical Analysis*

All values were expressed in terms of mean +/- SEM. Statistical analysis was done by analysis of variance, ANOVA. A value of $p < 0.05$ was taken as statistically significant.

**II. Results**

*DPPIV is Expressed in Endothelial Cells and Submucosal Seromucus Glands from Patients*

Using immunohistochemistry, DPPIV-like immunoreactivity (-LI) was detected in submucosal seromucus glands and leukocytes. In submucosal seromucus glands, DPPIV-LI appeared to be located in the apical position. Endothelial cells in blood vessels expressed weak DPPIV-LI. Some epithelial cells located in the human nasal mucosa were also DPPIV positive. However, these cells did not show any positive immunoreactivity for CD1A and Protein 100, suggesting they were not Langerhans cells.

*Correlation Between DPPIV and Inflammation in the Human Nasal Mucosa*

Nasal mucosa biopsies were sampled from both nostrils in 45 patients suffering from rhinosinusitis to determine if DPPIV activity was affected by the mucosal inflammation. DPPIV activity was found to vary from undetectable to 707 pmol/min/mg. Histological analysis revealed marked differences in the density of inflammatory cells within the submucosa of the nasal biopsies studied. A low activity of DPPIV was associated with a high density of inflammatory cells in the nasal mucosa of patients with chronic rhinosinusitis. When the density of inflammatory cells observed was plotted against DPPIV activity, the regressive correlation was found to be statistically significant ($p < 0.001$). Nasal mucosa samples obtained from smokers exhibited a significantly lower DPPIV activity than samples obtained from non-smokers having the same low density of inflammatory cells ($p < 0.01$).

When the density of inflammatory cells in each biopsy was plotted against the subjective evaluation of nasal obstruction, the data fitted a statistically significant correlation ($p < 0.01$), suggesting that nasal inflammation increased in parallel with subjective nasal obstruction severity. In addition, nasal mucosa biopsies were obtained from 10 patients with significant improvement of their symptoms ($p < 0.001$), 6 months after endonasal surgery. The DPPIV activity was significantly increased in all of the samples studied when compared to the preoperative state ($p < 0.001$) indicating that DPPIV activity can be restored when chronic rhinosinusitis is cured. Since there was a clear negative correlation between DPPIV activity and nasal mucosal inflammation, the hemodynamic effect of SP in the presence of recombinant DPPIV was studied in pig nasal mucosa.

*DPPIV Modulates Inflammatory Response Mediated by Histamine in Pigs*

Histamine is responsible for the early-phase allergic reaction and exerts both direct and indirect effects on sensory nerves, glands and blood vessels of the nasal mucosa (Alving, *Acta. Physiol. Scand. 597*:1-64 (1991)). There is direct evidence that histamine can release peptides from capsaicin sensitive sensory nerves in the lung by activation of $H_1$-receptors (Alving, *et al., Acta. Physiol. Scand. 138*:49-60 (1990)). In addition, endogenous or exogenous histamine stimulates sensory fibers, possibly by acting on specific receptors to increase the release of CGRP and SP (Tani, *et al., Neurosci. Lett. 115*:171-176 (1990).

Histamine i.a. local injection following sympathetic nerve stimulation (SNS) at 10 Hz for 5 minutes showed a significant reduction in duration at $5.4 \times 10^{-09}$ moles (33%) and at $1.4 \times 10^{-07}$ moles (32%) when compared to control conditions. After i.a. administration of 50 μg of DPPIV and SNS, histamine challenge showed no significant variation in the area under the curve (AUC) or vascular resistance (Vr) when compared to control. However, the duration of histamine effect was reduced by 33.7% at a dose of $5.4 \times 10^{-08}$ moles ($p < 0.05$) and by 50.7% at a dose of $1.4 \times 10^{-07}$ moles ($p < 0.01$). After phenoxybenzamine (PBZ) pretreatment, DPPIV and SNS, histamine vasodilatory effects were markedly reduced. When compared to control, the AUC showed a reduction of 81.9% at a dose of $5.4 \times 10^{-08}$ moles ($p < 0.001$) and a reduction of 81.8% at a dose of $1.4 \times 10^{-07}$ moles ($p < 0.001$). Vr showed a reduction of 67.9% at $5.4 \times 10^{-08}$ moles ($p < 0.01$) and a reduction of 61.1% at $1.4 \times 10^{-07}$ moles ($p < 0.05$). Duration of effect was also reduced at doses of $5.4 \times 10^{-09}$ moles (42.6%, $p < 0.01$); $5.4 \times 10^{-08}$ moles (55.6%, $p < 0.001$); and $1.4 \times 10^{-07}$ moles (62.5%, $p < 0.001$).

*DPPIV Modulates the Vasodilation Evoked by Capsaicin in Pigs*

Capsaicin (8-methyl-N-vanillyl-6-nonenamide), by activating unmylinated sensory C-fibers, acts in many respects like histamine. Capsaicin has been shown to release tachykinins (Hua, *et al.*, *Neurosci. 19*:313-319 (1986)) as well as CGRP (Stjarne, *et al.*, *Regul. Pept. 33*:251-262 (1991)) both *in vitro* and *in vivo*. Capsaicin injection following SNS showed a dose-dependent decrease of Vr similar that to observed under control conditions. The vasodilatory effect of capsaicin after SNS and PBZ pretreatment showed no significant variation at any doses. However, the duration of vasodilation evoked at $8.2 \times 10^{-08}$ moles was significantly reduced (39.9%, $p< 0.01$) and AUC was reduced by 26.6% at $8.2 \times 10^{-08}$ moles ($p< 0.05$). Capsaicin effects were also modified after DPPIV and SNS. The AUC observed at a dose of $8.2 \times 10^{-08}$ moles showed a reduction by 56.6% ($p< 0.01$). The Vr response was reduced by 34.8% ($p< 0.05$) and the duration of effect at doses of $3.3 \times 10^{-09}$ moles and $8.2 \times 10^{-08}$ moles was reduced by 55.4% ($p< 0.05$) and 50.5% ($p< 0.001$) respectively. After PBZ, DPPIV and SNS, there was marked reduction in Vr, duration of effect, and AUC. Vr at $8.2 \times 10^{-08}$ moles was reduced by 39.9% ($p< 0.01$). At $3.3 \times 10^{-09}$ moles and $8.2 \times 10^{-08}$ moles, the duration of the vascular response was reduced by 64.3% ($p< 0.05$) and 56.8% ($p< 0.001$). At $8.2 \times 10^{-08}$ moles, AUC was reduced by 66.7% ($p< 0.01$).

*DPPIV Modulates the Vasodilatory Response Mediated by Bradykinin in Pigs*

Bradykinin (BK) is a polypeptide involved in nociception and humoral regulation of vascular tonicity and permeability. BK produces marked vasodilation, increases capillary permeability and is involved in most inflammatory reactions, including rhinitis (Svensson, *et al.*, *J. Allergy Clin. Immunol. 85*:828-833 (1990)). Like histamine and capsaicin, BK stimulates nociceptive sensory nerves to produce CGRP and SP (Hua, *et al.*, *Can. J. Physiol., Pharmacol. 73*:999-1006 (1995); Vasco, *et al.*, *J. Neurosci. 14*:4987-4997 (1994)).

BK injection following SNS showed a dose-dependent decrease of Vr similar to that observed under control conditions. After DPPIV and SNS, BK showed only variation in the duration of effect at doses of $9.4 \times 10^{-11}$ moles (34%, $p< 0.01$) and $9.4 \times 10^{-09}$ moles (38.3%, $p< 0.001$). After PBV, DPPIV and SNS, vascular parameters were reduced to a smaller extent than that seen with histamine and capsaicin. For example, AUC after i.a. administration of BK was reduced by 29.3% at $9.4 \times 10^{-09}$ moles ($p< 0.01$) and by 40% at $9.4 \times 10^{-11}$ moles ($p< 0.01$). At $9.4 \times 10^{-10}$ moles duration of effect was reduced by 32.2% ($p< 0.05$) and at $9.4 \times 10^{-09}$ moles, duration was reduced by 39.4% ($p< 0.001$).

*DDPIV Modulates the Vasodilatory Response Mediated by SP in Pigs*

Repeated injection of high doses of SP caused a reproducible decrease in sphenopalatine vascular resistance, indicating that the desensitization of neurokinin (NK) receptors did not occur. Therefore, the same animal was used to perform dose-response curves with SP both before and after pretreatment with DPPIV. A dose-response curve for duration of vasodilation and area under the curve was constructed using doses of SP in the range of 1 pg to 0.1 µg. A reproducible dose-response curve was obtained over a 45 minute period using 6 doses of SP. Vasodilation ranged from 15.1% +/- 3.3% to 37.3% +/- 3.3% after local intraarterial administration of 1 pg and 100 ng of SP respectively. To determine whether vasodilation evoked by SP was affected by pretreatment with DPPIV, two doses of SP (100 pg and 1 ng) were administered and a reduction in SVR of 13.1% and 14.9% was observed. The same animals received 50 µg (530 pmoles) of DPPIV and similar doses of SP were injected. Recombinant DPPIV had no vascular effect per se. Subsequent administration of the SP resulted in a dramatic diminution of SP-evoked vasodilatory response (by 66 and 71% respectively) when compared to controls. Since DPPIV cleaves SP into SP 5-11, experiments were conducted to determine whether SP 5-11 is also capable of decreasing SVR. Similar to what was observed with respect to SP, a dose response curve was obtained with 10 pg to 1 µg of SP 5-11 (5 to 35% decrease in SVR respectively). However, the vasodilatory effect of SP 5-11 was considerably lower when compared to SP. Based on molarity, SP is 200 fold more potent than SP 5-11 as a vasodilator. Therefore, DPPIV administration to pigs might result in an almost immediate conversion of SP 5-11.

To further characterize the nature of neurokinin receptor subtype involved in the reduction of SVR, pigs were pretreated with a NK1 antagonist, L-733060, at 114 nmoles/kg to evaluate the blockade of SP-induced decreased in SVR. It was found that the antagonist has a vasoconstrictive effect. A clear inhibition of the SP effect was observed at all doses of SP used except 100 ng. Thus, the SP effect on SVR is likely to mediated by the NK1 receptor.

## III. Discussion

The localization of the enzyme DPPIV in the apical position of nasal exocrine cells in seromucus glands suggests a role of this enzyme in the protective function of nasal mucus.

The presence of DPPIV in vascular endothelial cells and T-cells is in line with earlier histochemical studies of DPPIV in mammalian tissue (Sannes, *J. Histochem. Cytochem. 31*:684-690 (1983)) as well as with more recent reports on the distribution of human DPPIV (Van. Der. Elden, *et al.*, *Clin. Exp. Allergy 28*:110-120 (1998)). DPPIV immunoreactivity was also observed in some intraepithelium cells of nasal mucosa. These cells were probably not Langerhans cells since they did not express CD1A or Protein 100 immunoreactivity on their surface.

Correlation between DPPIV activity and inflammation of nasal mucosa showed a marked decrease of enzyme activity in the presence of severe inflammation. In agreement with Van der Velden, *et al.* (*Id.*), who showed that DPPIV activity is reduced in the serum of healthy smokers compared to non-smokers, DPPIV activity was reduced in nasal samples with severe inflammation. In contrast, high DPPIV enzymatic activity was correlated with a small number of inflammatory cells in the nasal mucosa. The fact that enzyme activity increased after treatment of chronic rhinosinusitis is also consistent with the involvement of the enzyme in this pathology. Pretreatment with 50 ug of DPPIV significantly decreased duration of vasodilation and spenopalatine artery vascular resistance when DPPIV was used alone or with the adrenoreceptor blocker phenoxybenzamine.

DPPIV significantly decreased both peak and duration of the vasodilation evoked by SP. It was found that the SP 5-11 was at least 100 fold less potent than SP at causing vasodilation. These results suggest that a lost of DPPIV expression occurs during chronic rhinosinusitis and that the resulting reduction of SP degradation contributes to the maintenance of nasal mucosa inflammation. In this regard, Nieber, *et al.* have shown that allergic patients have higher baseline levels of SP-ir in nasal lavage fluids than non-allergic controls and that intranasal allergen increases SP levels in nasal lavage fluids only in subjects with grass pollen allergy (*J. Allergy Clin. Immunol. 4 pt1*:646-652 (1992)). The antibody used for the SP assay in the Nieber study required both an intact N- and C-terminus peptide sequence. The data is in line with that presented herein and could be explained by the fact that allergic patients expressing less DDPIV than normal volunteers produce intact SP which could be measured by their antibody. In contrast, normal volunteers cleave their SP into SP 5-11 fragments which are no longer detected in the SP assay. SP effects on the vascular bed of the nasal mucosa are maintained only when allergenic stimulation persists in subjects with nasal allergies (*i.e.*, subjects without DPPIV).

In parallel to SP cleavage by DPPIV into SP 5-11, a N-terminal fragment, SP 1-4, is generated and has been reported to exert an antagonist affect on SP action (Sakurada, *et al.*, *J. Pharm. Sci. 88*: 1127-1132 (1999)). Thus, DPPIV might modulate SP action, not only by producing a less active NK1 agonist, but also by interfering with SP effects. Since SP 5-11 (but not SP) is subject to cellular uptake, DPPIV may also act to terminate the action of SP via conversion to a form which is removed by cellular processes (Nakata, *et al.*, *J. Neurochem. 37*:1529-1534 (1981)). Once SP is cleaved by DPPIV into SP 5-11, this fragment is further processed with a high efficiency by aminopeptidase M into inactive fragments. Thus, DPPIV first converts SP to a much less active form, SP 5-11, which is then completely inactivated by the action of aminopeptidase M. Finally, DPPIV could potentially act upon other peptides which are known to be involved in promoting inflammation and which have structures that lend themselves to degradation by this enzyme.

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All references cited are fully incorporated by reference. Having now fully described the invention, it will be understood by those of skill in the art that the invention may be performed within a wide and equivalent range of conditions, parameters and the like, without affecting the spirit or scope of the invention or any embodiment thereof.